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(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Emanuele Ostuni et al.
Serial No.:	10/668,679
Confirmation No.:	1051
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For:	SELECTIVE DEPOSITION OF MATERIALS ON CONTOURED SURFACES
Examiner:	D. M. Naff
Art Unit:	1651

Certificate of Mailing Under 37 CFR 1.8(a)
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the U.S. Postal Service on the date shown below with sufficient postage as First Class Mail, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: Jan. 3, 2007 Signature

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME CITED PATENT OR PUBLICATION UNDER 37 C.F.R. §1.131

Sir:

We, Emanuele Ostuni, Christopher S. Chen, Donald E. Ingber, and George M. Whitesides, declare that:

1. This Declaration is to establish completion of the invention as recited in at least claims 1-24 and 26-27.

2. We are the inventors of the above-identified application and of the claims specifically mentioned in Paragraph 1.

3. The reduction to practice of claims 1-24 and 26-27 took place in the United States prior to March 17, 2000, the effective filing date of U.S. Patent No. 6,893,850 to Ostuni, et al. which was cited in the office action mailed July 3, 2006.

4. Relevant to the reduction to practice discussed in Paragraph 3, prior to March 17, 2000, we performed experiments described below, in a manner substantially similar to that described in the examples of the present application. We submit, herewith, copies of excerpts from an inventor's laboratory notebooks documenting the experiments, as Exhibits A and B. The dates on the notebook pages have been redacted in these Exhibits. Exhibits A and B illustrate methods representative of those described in the above-identified application and embraced by certain claims thereof. The experiments were performed by us or under our supervision and control in the United States prior to March 17, 2000.

The methods performed and illustrated in Exhibit A comprise different conditions for selectively depositing materials such as proteins and cells on substrates having contoured surfaces including protrusions and recesses. Substrates were formed in PDMS by first preparing a master including posts (e.g., protrusions) of photoresist supported on a silicon wafer prepared by photolithography (claims 17-19). The height of the post, which corresponded to the depth of the recesses (or microwells), was controlled by the choice of the photoresist and spinning rate. The mask used for photolithography had posts with diameters of 3 and 5 microns (e.g., microprotrusions) spaced by recesses having widths of 8 and 10 microns (claims 9-16, 20-23, 26). The PDMS was cured against these masters at 60 ° C for 2 hours and peeled away from the silicon wafers to give a contoured surface including an array of protrusions molded into the surface of the polymer. Substrates were then cut to the desired size (typically a few centimeters in length and width) and washed with ethanol and distilled water before use in the cell culture.

Delivery of BSA (e.g., a protein that is cytophobic, claims 4 and 6) to the top surfaces of the protrusions, and of fibronectin (FN, e.g., a protein that is cytophilic, claims 5 and 7) to the recesses, was accomplished using a two-step procedure. This procedure is depicted in the right-most figure of Exhibit A. A PDMS substrate was placed inside a sterile Petri dish. In the first step, a drop of BSA (10 μg/mL, e.g., a first fluid having an advancing contact angle of greater than about 90°,

claim 24) in phosphate buffered saline (PBS) buffer was placed on top of the substrate for thirty minutes to allow the protein to deposit on the top surfaces of the protrusions. The liquid trapped air bubbles inside the recesses; these air bubbles protected the interior surface of the recesses from contact with the solution containing BSA (claims 1, 26, and 27). Care was taken to avoid shaking or vibrating to avoid dislodging these bubbles. The substrate was then rinsed gently with PBS three times. Brief exposure of the substrate to house vacuum assured that all the bubbles escaped from the recesses. The buffer was then exchanged with a solution of FN (e.g., a second fluid) by aspirating the buffer using vacuum and placing a drop of solution of FN (50 μg/mL) on top of the array of protrusions. During this procedure, drying of the sample was avoided to prevent the formation of bubbles and damage to the adsorbed BSA. The adsorption of the FN to the recesses of the substrate was then allowed to proceed at room temperature for one hour (claim 2).

To pattern cells on the substrates, the cells were first prepared using the following procedure. Bovine adrenal capillary endothelial (BCE) cells were cultured under 10% CO_2 on Petri dishes coated with gelatin in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% calf serum, 2 mM glutamine, 100 μg/mL streptomycin, 100 μg/mL penicillin, and 1 ng/mL basic fiber glass growth factor (bFGF). Prior to incubation with the substrates prepared using the method described above, the cells were dissociated with culture plates with trypsin-EDTA and washed in DMEM containing 1% BSA (BSA/DMEM). The cells were then placed on the substrate in a chemically defined medium (10 μg/mL high density lipoprotein, 5 μg/mL transferin, 5 ng/mL bFGF in BSA/DMEM, e.g., a third fluid) and incubated in 10% CO_2 at 37 °C. These cells attached only to surfaces that were treated with FN (e.g., the recesses using the procedure described above) and were absent from areas patterned with BSA (e.g., the surfaces of the protrusions) (claims 3 and 8). The cells were incubated for 4 hours, and were routinely cultured for up to 48 hours on the substrate.

To stain the cells, the substrates coated with FN were exposed to 4% paraformaldehyde (v/v) in PBS buffer (PFA) for 20 min, washed with PBS, and then immersed in a solution of rabbit anti-human fibronectin IgG (Sigma, 5 μg/mL) for 1 hour. The substrates were rinsed twice with PBS containing 0.1% (w/v) BSA and 0.1% (w/w) Triton X-100, placed in contact with 100 μL of Texas Red®-labeled goat anti-rabbit IgG (Amersham Life Sciences, 50 μg/mL) for 1 hour, rinsed, and then mounted with Fluoromount-G (Southern Biotechnology, Inc.) onto microscope slides.

Substrates that contained cells patterned on FN in the recesses were fixed with PFA for 20 min, washed with methanol for 1 min, and stained with Coomassie Blue (5 mg/mL in 40% v/v methanol, 10% v/v acetic acid, and 50% v/v water) for 30 sec; they were then rinsed with distilled water and dried in air.

In order to pattern cells onto surfaces of the protrusions (instead of surfaces of the recesses), a procedure similar to the one described above was followed except FN was first delivered to the top surfaces of the protrusions, and then BSA was delivered to the surfaces of the recesses. This procedure is depicted in the left-most figure shown in Exhibit A. Cells were attracted to the surfaces coated with FN (e.g., the top surfaces of the protrusions) and were absent from areas patterned with BSA (e.g., the surfaces of the recesses).

Exhibit B shows further experiments of selectively depositing proteins and cells on substrates having contoured surfaces including protrusions and recesses.

5. All statements made herein of our own knowledge are true, and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like made in a declaration are punishable by fine or imprisonment or both under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Full name of undersigned inventor: Emanuele Ostuni

Signature: _____

Date: _____

Citizenship: Italy

Residence: 175 Fayetter Street, Watertown, MA 02472

Full name of undersigned inventor: Christopher S. Chen

Signature: _____

Date: _____

Citizenship: U.S.

Residence: 301 Warren Avenue, Baltimore, MD 21205

Full name of undersigned inventor: Donald E. Ingber

Signature: _____

Date: _____

Citizenship: U.S.

Residence: 71 Montgomery Street, Boston, MA 02116

Full name of undersigned inventor: George M. Whitesides

Signature: _____

Date: _____

Citizenship: U.S.

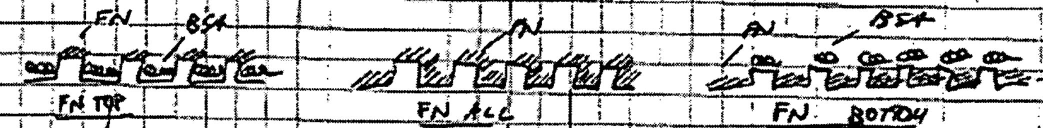
Residence: 124 Grasmere Street, Newton, MA 02158

EXHIBIT A

Experiments w/ BCE cells on PDMS posts

· PDMS posts were 3 & 5 μm diameter spaced by 8 & 10 μm. Both types were 3 μm tall

· 3 conditions



FN TOP

· Drop of FN (50 μg/ml) on sample for 1 hour

· PBS rinse

· Drop of BSA (10 mg/ml) for 30 min → Vacuum

FN ALL

· Drop of FN (50 μg/ml) vacuum

· 1 hour incubate

FN BOTTOM

· Drop of BSA (10 mg/ml) for 30 min ~~PBS~~

· PBS Rinse

· Drop of FN (50 μg/ml) for 1 hour after vacuum

· Block w/ DMEM

· 3 dishes: 2 samples in each

400000 cells each dish

· Cells seem to attach on ϕ.

3 μm posts regions have a lot of damage

· 2 pictures taken of FN ALL 5(x) : EOTOPOFPOSTS; EOTOPOFCELL

· FN BOTTOM: few cells seem dead and poor adhesion

EXHIBIT B

Experiments on cells in parallel.

→ Using standard method

- 2x 25(5) ; 2 x 50(8) ; 2x 50(1.3) each are labelled A or B
- place 300 µl BSA (2% ETOH; PBS) on top for 3 hours incubation
- B look better under scope those wells filled w/ air.
- Rinsed w/ 2% ETOH (PBS) by wounding drop & then rinse w/ PBS extensively → fill; decant, fill; decant; fill followed by 2 short (~ 30 sec.) vacuum cycles w/ tapping to get rid of bubbles. Under scope look filled w/ liquid. Then aspirate liquid & add FN (50 µg/ml in PBS) for a 2 hour incubation.
- 50(5) A Some dewetting occurred at BSA step for the same wells filled
- 25(5) B BSA dried for ca. 10 sec. before FN step

- B samples were then used in cell culture. Blocked w/ DMEM 1% BSA for 15 minutes then 4 ml 1% BSA, .5 ml 10 x defined media, + .5 ml cells (500000/ml)

- A samples were rinsed w/ PBS 2x then fixed w/ PFA for 20 minutes. Anti human for 45 minutes (1:100 dilution) 2X rinse w/ PBS (.1% BSA; .1% Triton X); 45 minutes anti rabbit - Texas red. Stored w/ .01% Azide.

B Samples

After 12 hours in all culture samples were fixed w/ PFA for 20 minutes & stained w/ coomassie blue for ca. 20 seconds.

- .50(1:3) Cells pattern beautifully but don't spread out to full diameter of wells
  Density of patterning is fairly high
- .50(5) Cells in wells w/ lower density. Patterning is not very regular
- .25(5) Since BSA dried a little more, cells find areas between wells, but many cells in single wells

A Samples → Fluorescence

- .50(1:3) FN patterns only in wells w/ little bits
- .50(5) FN seems to be just on edges of wells
- .25(5) FN patterns beautifully just in wells